The Effect of Indomethacin Blockade of Prostaglandin Synthesis on Blood Pressure of Normal Rabbits and Rabbits with Renovascular Hypertension

J. CARLOS ROMERO AND CAMERON G. STRONG

With the technical assistance of Sharon M. Schryver, V. Ray Walker, and David C. Manahan

SUMMARY Indomethacin inhibits the synthesis of prostaglandin and the release of renin. These effects were studied in normal rabbits and rabbits with two-kidney Goldblatt hypertension (2KGH) and one-kidney Goldblatt hypertension (1KGH) by giving daily intravenous injections of indomethacin (3 mg/kg after two initial doses of 9 mg/kg), and in appropriate control rabbits given diluent phosphate buffer without indomethacin. In normal rabbits, indomethacin significantly decreased immunoreactive plasma prostaglandin E-like substance (IPGE) and plasma renin activity (PRA). Indomethacin did not change plasma creatinine (PCr) or mean blood pressure but it decreased renal blood flow (RBF) and glomerular filtration rate (GFR). In 2KGH rabbits, responses depended on the level of renal function and, to a lesser extent, on the level of PRA. In six of 10 2KGH rabbits in which hypertension developed without significant changes in PRA, IPGE, PCr, RBF, and GFR, indomethacin produced changes similar to those seen in normals. In the other four rabbits, development of 2KGH was accompanied by increased PRA, increased IPGE, and decreased RBF and GFR, and indomethacin produced renal failure, oliguria, malignant hypertension, and death within 5 days. In 1KGH rabbits, indomethacin decreased IPGE, PRA, and renal function but increased mean blood pressure. These observations suggest that prostaglandins exert a protective effect on renal function in renovascular hypertension.

RECENT evidence suggests that the vasodilator and natriuretic actions of prostaglandins and their inhibitory effect on adrenergic activity1 may play important roles in the homeostatic regulation of renal blood flow and arterial blood pressure. In agreement with this line of thought, a hypothesis has been advanced suggesting that renal ischemia subsequent to a deficient synthesis of prostaglandin in the kidney may induce hypertension secondary to salt retention and stimulation of renal pressor principles.2 This concept has been supported by preliminary reports showing that the blockade of prostaglandin synthesis by indomethacin results in a significant increase in blood pressure in normal rabbits3 and aggravates renovascular hypertension in rats.4 In these reported studies, changes in plasma renin activity and renal function were not measured. Such measurements might have allowed a better understanding of the mechanism underlying the elevation in blood pressure.

From the Mayo Clinic and Mayo Foundation, Rochester, Minnesota. Supported in part by Research Grant HL-14496 of the National Institutes of Health, Public Health Service.

Dr. J.C. Romero is an Established Investigator of the American Heart Association.

Address for reprints: Dr. J.C. Romero, Mayo Clinic, 200 First Street, S.W., Rochester, Minnesota 55901.

Received April 2, 1976; accepted for publication August 18, 1976.
BLOOD SAMPLING AND PREPARATION OF PLASMA FOR DETERMINATION OF PRA, IPGE, AND PCR.

During the 6-day control period and during the 35 days that followed the surgical procedure, 6-ml blood samples for determination of PRA, IPGE, and PCR were obtained every week. Blood samples were also drawn 9 hours after treatment with indomethacin began and daily or every other day during the 10 days of treatment. The samples were drawn between 8:00 a.m. and 9:00 a.m. from a 21-gauge needle inserted in the central artery of the ear. The first 2 ml were collected in a separate tube for determination of PRA, whereas the remaining 4 ml were collected in different tubes for determination of IPGE and PCR. This was done to avoid an artificial increase in PRA induced by blood sampling. Blood samples for determination of PRA were centrifuged, and plasma was separated and kept frozen until determination by radioimmunoassay. A similar procedure was followed for PCR. Plasma samples for determinations of IPGE were separated after refrigerated centrifugation and were frozen immediately at 0°C until extraction. Plasma (1 ml) was acidified to a pH between 3 and 4 with 1 N HCl; 4 ml of redistilled ethyl acetate. Both extractions were then combined and flash evaporated (Buchler-Rotary Evapormix).

Column chromatography was performed according to the procedure recommended by Caldwell et al. After separation of IPGE and conversion to prostaglandin B (PGB) by alkaline treatment, PGB was radioimmunoassayed using labeled PGB, and anti-PGB antibodies (Clinical Assays). Free-labeled PGB was separated from PGB bound to antibodies with charcoal. Final values are expressed in nanograms per milliliter of plasma. Recoveries from extraction and column separation obtained with 3H-labeled prostaglandin E2 (H-PGE2) (New England Nuclear) in 30 samples from normals, 30 samples from 2KGH, and 30 samples from 1KGH ranged from 50% to 70%. Conversion of PGE to PGB in these samples ranged from 90% to 95%.

Renal clearances of p-aminohippurate (PAH) and insulin (In) were performed on the 6th day of the control period, on the 35th day after the development of hypertension, and on the 5th and 10th days of treatment with indomethacin. This was done by giving the priming and maintenance dose of In and PAH through the marginal vein of the right ear. Blood samples (3 ml) for determination of In and PAH were obtained through a 21-gauge needle inserted in the central artery of the ear. In general, the procedure was identical to that used in previous studies, with the exception that urine samples were collected through a 7-Fr. catheter inserted into the bladder through the urethra rather than by catheterizing the ureters.

During the experiment, blood pressure was recorded with a modification of the Grant-Rothschild capsule except during the 9 hours that followed the first two injections of indomethacin when blood pressure was recorded on a polygraph (Grass model 7) connected to a transducer (Statham DB23) that sensed pressure through a 21-gauge butterfly needle placed in the central artery of the ear. This was done to detect acute changes in blood pressure. PCR levels were estimated by a modified picric acid method used in previous studies.

PRA was measured by radioimmunoassay according to the procedure of Haber et al. Anti-angiotensin I antibodies were obtained from New Zealand rabbits treated with serum albumin-angiotensin I complex prepared according to the procedure of Goodfriend et al. Angiotensin I labeled with 125I and inhibitors of the converting enzyme angiotensinase were purchased from Squibb. The reproducibility of this method has been published elsewhere.

STATISTICAL ANALYSIS

The significance of differences in changes between the groups or occurring within one group at different times was analyzed by the unpaired and paired t-test. When the variances between the two groups were unequal, treatment differences were analyzed by a rank-sum test.

**Results**

**EFFECT OF INDOMETHACIN ON BLOOD PRESSURE, PRA, IPGE, AND RENAL FUNCTION OF NORMAL RABBITS**

During the control period, as well as during the 35 days preceding treatment with indomethacin, blood pressure, PRA, RBF, PCR, and IPGE remained constant in normal rabbits (Fig. 1). The administration of indomethacin was followed during the next 24 hours by a significant (P < 0.01) decrease in the mean values of IPGE. On the 10th day of treatment, these mean values returned to approximately 50% of the control values.

Changes in IPGE were accompanied by a significant (85%) (P < 0.01) decrease in the mean value of PRA, and this decrease remained during the 10 days of treatment. On the contrary, no significant changes were detected in blood pressure during the first 5 days of treatment; later, the blood pressure decreased slightly. The total decrease from the 5th day to the 10th day was 12 mm Hg (P < 0.05).

On the 10th day, indomethacin induced moderate but significant (P < 0.05) decreases in RBF (from 40.4 ± 3.3...
to 33.9 ± 1.3 ml/min and GFR (from 8.2 ± 0.6 to 5.1 ± 0.5 ml/min) (Fig. 1). PCr levels did not change significantly.

The administration of phosphate buffer without indomethacin to four normal rabbits did not result in any significant change in any of the parameters studied (Table 1).

Histological examination of the kidneys removed from the normal rabbits that were treated showed localized areas of interstitial and periglomerular fibrosis infiltrated with inflammatory cells. These lesions were absent in the nontreated rabbits.

EFFECT OF INDOMETHACIN ON BLOOD PRESSURE, PRA, IPGE, AND RENAL FUNCTION ON 2KGH RABBITS

In six rabbits the clipping of one renal artery caused a significant (P < 0.01) increase in mean arterial pressure of 36 ± 3 mm Hg but no significant change in PRA, RBF, IPGE, or PCr (Fig. 2). In four other rabbits the renal arterial constriction elicited a much greater increase in mean arterial pressure. The increase in blood pressure in these four rabbits was accompanied by a significant increase (P < 0.05) in PRA and IPGE. These four rabbits also showed significant reductions (P < 0.01) in RBF.

The response to indomethacin was different in these two groups of rabbits. In the group of six rabbits in which hypertension evolved without changes in RBF, indomethacin treatment was followed by a series of changes that resembled those noted in normal rabbits, in that the initial decrease in circulating prostaglandin was accompanied by a marked decrease in PRA with no increment in blood pressure. These rabbits also exhibited a transient decrease in RBF (from 40.0 ± 1.4 to 26.8 ± 1.8 ml/min) and GFR (from 8.5 ± 0.4 to 5.4 ± 0.1 ml/min) on the 5th day of treatment. However, PCr remained unaltered during the treatment.

In the four 2KGH rabbits that had decreased RBF in response to clipping, treatment with indomethacin caused renal failure with a progressive increase in PCr, oliguria, and malignant hypertension and a progressive increase in PRA. These rabbits did not survive for more than 5 days.

All these described changes induced by indomethacin in 2KGH rabbits were not observed in the control 2KGH untreated group in which the administration of phosphate buffer without indomethacin did not result in any significant change in BP, PRA, IPGE, or PCr (Table 1).

The histological appearance of kidneys from six 2KGH rabbits treated with indomethacin revealed the existence of interstitial and periglomerular fibrosis and infiltration with chronic inflammatory cells similar to those found in the normal group that was treated. These lesions were more frequent in the contralateral than in the clipped kidney, and grossly the involvement of the renal parenchyma was greater than that seen in the normal group that was treated. On the contrary, the occurrence of interstitial and periglomerular fibrosis was rare in the group of untreated 2KGH rabbits.

Unfortunately, the sudden death of 2KGH rabbits precluded a systematic histological evaluation, but the kidneys obtained from two of these rabbits, 24 and 36 hours after the development of malignant hypertension, showed microscopic lesions that resembled those seen during the development of experimental vasomotor acute renal fail-

Table 1 Normal and Hypertensive Rabbits Treated With Phosphate Buffers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal control rabbits (n = 4)</th>
<th>2KGH rabbits (n = 4)</th>
<th>1KGH rabbits (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control, 6th day</td>
<td>After clipping, 35th day</td>
<td>PO4-buffer treatment, 10th day</td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td>72 ± 3</td>
<td>74 ± 2</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>16 ± 4</td>
<td>20 ± 3</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>IPGE (ng/ml)</td>
<td>1.3 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>40 ± 2.7</td>
<td>38 ± 3.1</td>
<td>39.1 ± 1.0</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>7.4 ± 0.4</td>
<td>7.8 ± 0.4</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>PCr (mg/dl)</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se.

2KGH = two-kidney Goldblatt hypertension; 1KGH = one-kidney Goldblatt hypertension; BP = blood pressure; PRA = plasma renin activity; IPGE = prostaglandin E; RBF = renal blood flow; PCr = plasma creatinine.
FIGURE 2 Effect of indomethacin on 2KGH rabbits. Six rabbits (closed circles, solid bars) showed changes similar to those seen in normal rabbits. Four rabbits (open circles, open bars) showed increased PRA and IPGE with decreased RBF during development of 2KGH; in these rabbits, indomethacin produced renal failure, malignant hypertension, and death within 5 days. 2KGH = two-kidney Goldblatt hypertension; other abbreviations as in Figure 1.

FIGURE 3 Effect of indomethacin on 1KGH rabbits. PRA and IPGE were reduced, hypertension was aggravated, RBF was decreased, and plasma creatinine was increased by indomethacin treatment. 1KGH = one-kidney Goldblatt hypertension; other abbreviations as in Figure 1.

The major finding of this study is that the blockade of prostaglandin synthesis by the administration of indomethacin to normal rabbits and to 2KGH and 1KGH rabbits is not accompanied by any significant increase in blood pressure unless the treatment induced a significant reduction in the renal function. Our data also showed that renal failure did not occur in a consistent fashion, occur-
ring only in rabbits that exhibited an RBF below normal at the time treatment with indomethacin began. These findings strongly suggest that the elevation of blood pressure produced by indomethacin is not a phenomenon strictly related to the blockade of prostaglandin synthesis but one indirectly mediated by a decrease in renal function.

Therefore, it is relevant to analyze why indomethacin induced renal failure only in rabbits that already had a significant decrease in RBF and to evaluate whether there is an identifiable factor common to the production of renal failure and the aggravation of hypertension.

Previous studies have shown that the reduction of RBF induced by mechanical occlusion of the renal artery or by infusion of angiotensin II elicits the release of prostaglandin E. These findings suggested that the vasodilator action of renal prostaglandin E could be important as a local regulator in minimizing renal ischemia. This concept has received further support from other investigators who have shown that prostaglandin may be responsible for the reactive hyperemia that occurs after the temporary occlusion of the blood supply to the kidney and to other tissues such as skeletal and cardiac muscle. If the enhanced synthesis of prostaglandin which follows renal ischemia is chronically operative, then it could be assumed that the remaining RBF in kidneys in which the circulation was severely impaired by the narrowing of the renal artery (such as the clipped kidney of 2KGH and 1KGH rabbits) or by the vasoconstrictor action of excessive amounts of circulating angiotensin (such as the contralateral kidney of rabbits with 2KGH and high levels of PRA) is primarily maintained by an enhanced synthesis of prostaglandin. Hence, blockade of prostaglandin synthesis in these kidneys would lead to a significant reduction in RBF and renal failure. In contrast, such effects are not seen when the renal circulation is not severely impaired (as in normal rabbits or in 2KGH rabbits that have normal RBF). The validity of these assumptions is further supported by the finding in previous studies that the decrease in RBF produced by indomethacin is proportional to the previous decrement in RBF and indicates that the importance of renal prostaglandins in maintaining RBF increases with the degree of renal ischemia. Furthermore, the concept that the enhanced synthesis of renal prostaglandin plays an important role in the maintenance of the remaining RBF when renal circulation is compromised also comes from the previous finding that the blockade of prostaglandin synthesis with indomethacin aggravates the course of glycerol-induced (vasomotor type) renal insufficiency in which the major initial pathological event is believed to be an exaggerated constriction of the glomerular afferent arterioles. In contrast, indomethacin does not aggravate the course of the nephrotoxic type of acute renal failure induced by mercuric chloride in which the primary event is tubular damage. Thus, the histological appearance of renal tubular and glomerular ischemic lesions and renal infarction seen in kidneys from rabbits with severe 2KGH and 1KGH might be caused by the cessation of the vasodilator protective effect of prostaglandins.

In analyzing the consequences derived from the blockade of prostaglandin synthesis, one should consider the difficulties in ruling out the possibility that such effects were produced directly by indomethacin. These drawbacks have been partially overcome by other investigators who determined the reproducibility of the observed effect when the blockade of prostaglandin synthesis was induced by other aspirin-like substances such as meclofenamate. These uncertainties cannot be resolved by the results of this study, which was designed to define the mechanisms underlying the reported elevation in blood pressure when the synthesis of prostaglandin is blocked with indomethacin. Within this context, renal insufficiency followed the treatment with indomethacin and was observed only in rabbits that already had a significant decrease in RBF. Because blood pressure increased only in rabbits that developed renal insufficiency, blood pressure should be analyzed further to determine to what extent further increases were conditioned by failure in renal function. If the blood pressure of 1KGH rabbits was volume-dependent and they developed progressive renal failure with a urinary output significantly lower than that found in 1KGH untreated control rabbits, it would be logical to assume that the extracellular fluid volume of these rabbits was expanded during the treatment with indomethacin and that this expansion contributed to the aggravation of hypertension.

The aggravation of hypertension induced by indomethacin in 1KGH rabbits was also accompanied by an upward trend in PRA (Fig. 3). However, several factors tend to abrogate its importance in the aggravation of hypertension: first, the maintenance of one-kidney hypertension is relatively independent of changes in PRA; second, in these same rabbits, one-kidney hypertension occurred without significant change in PRA; and third, the significant decrease in PRA which followed the treatment with indomethacin was not accompanied by any change in blood pressure.

In analyzing the factors responsible for blood pressure elevation in 2KGH rabbits that developed malignant hypertension during indomethacin treatment, one should consider that this type of hypertension is highly dependent on the levels of PRA. In fact, the simultaneous changes in blood pressure and PRA exhibited by the four rabbits from the beginning of the experiment support this concept. The development of two-kidney hypertension was paralleled by an increase in PRA; the decrease in PRA induced by indomethacin was accompanied by a transient decrease in blood pressure, and with the development of the malignant phase, both blood pressure and PRA increase. In these rabbits there also was a positive relationship between the increments in PCr and in blood pressure, and during this period they experienced significant oliguria. Therefore, expansion of the extracellular fluid volume could have potentiated the pressor effect of renin. An interesting observation made on the four rabbits with severe 2KGH was that malignant hypertension developed in the presence of levels of renin which were not higher than those recorded before treatment began. If one considers that other investigators have shown that indomethacin potentiates the vasopressor responses to catecholamines and angiotensin, one might assume that
in these four rabbits the vasopressor action of angiotensin in normal rabbits, the blockade of prostaglandin synthesis produced by indomethacin was a significant decrease in PRA. This confirms the previous observations of our group. From these experimental studies designed to elucidate the mechanism of the lowering effect of indomethacin on PRA, we concluded that such a phenomenon is not due to the interference by this drug with the renin-angiotensinogen reaction but rather to the blockade of renin release. If a decrease in renin release, and thereby in PRA, is due entirely to indomethacin and is not related to the blockade of prostaglandin synthesis, then it could be theorized that indomethacin is not the drug of choice to investigate the effect of prostaglandin withdrawal on blood pressure. The elevation in blood pressure expected after the disappearance of the vasodilator action of prostaglandin would be blunted by the depressive side effect of this drug on renin release. However, if the decrease in renin release is inherent in the blockade of prostaglandin synthesis and the withdrawal of prostaglandin is physiologically accompanied by a reduction of PRA, then the role of prostaglandin in the regulation of blood pressure will have to be considered within the context of the parallel changes elicited in the renal pressor system. The latter speculation may be correct, since attempts to separate the effects of blockade of prostaglandin synthesis from those exerted on renin release by the use of other blockers of prostaglandin synthesis with a molecular configuration different from that of indomethacin (such as meclofenamate and aspirin) have been negative so far. All these considerations are important because they restrict our experimental design in examining the cause of indomethacin enhancement of blood pressure reported by other investigators to experimental circumstances in which the blockade of prostaglandin will predictably be accompanied by a decrease in PRA.

In summary, the data obtained from this study show that the effect on blood pressure of the blockade of prostaglandin synthesis produced by indomethacin in normal rabbits and in renovascular hypertensive rabbits is conditioned by renal function. In 2KGH and 1KGH rabbits with severe impairment in renal circulation, the administration of indomethacin caused renal insufficiency and increased hypertensive and this could be attributed largely to either volume expansion or a further increase in PRA.

In renal vascular hypertensive rabbits with normal renal circulation and in normal rabbits, the blockade of prostaglandin synthesis produced by indomethacin is followed by a significant and sustained decrease in PRA and by no major changes in blood pressure.

Acknowledgments

Indomethacin was generously donated by Merck Sharp & Dohme, West Point, Pennsylvania.

References

27. Ames RP, Borkowski AJ, Siekmans AM, Laragh HI: Prolonged infusions of angiotensin II and norepinephrine and blood pressure, elec-
Recuperative Potential of Cardiac Muscle following Relief of Pressure Overload Hypertrophy and Right Ventricular Failure in the Cat

RICHARD L. COULSON, SHAHRIAR YAZDANFAR, EMIR RUBIO, ALFRED A. BOVE, GERALD M. LEMOLE, AND JAMES F. SPANN

SUMMARY This study examined the recuperative potential of cat hearts subjected to experimental right ventricular pressure overload (for a 10- to 14-day period) which provoked hypertrophy with and without congestive heart failure. Five groups of cats were studied: normal controls; one group with 70% pulmonary artery constriction which produced right ventricular hypertrophy (RVH); one group with an 87% constriction which also produced right ventricular hypertrophy but with congestive heart failure (CHF); and two groups which had been similarly subjected to pressure overload but which had been allowed a recovery period of 30 days after relief of the pressure overload. Both the 70% and 87% pulmonic constrictions were associated with extensive right ventricular hypertrophy, depression of myocardial contractile function, and severe reduction of cardiac norepinephrine stores (normal, 1.42 μg/g; RVH, 0.11 μg/g; CHF, 0.01 μg/g). After a 30-day period of relief from the pulmonic constriction normal hemodynamic function returned. In cats in which RVH had been relieved, right ventricular weight and contractile function were normal but catecholamine depletion persisted. Cats with relieved CHF showed depressed contractile function and depleted myocardial norepinephrine, and the right ventricular weight did not return to normal. Cardiac muscle of all pressure-overloaded nonrelieved hearts showed depressed velocity of shortening and depressed ability to sustain load. Cats with RVH alone regained normal muscle shortening velocity and load-bearing ability after relief. However, cardiac muscle from the CHF-relieved group recovered only unloaded shortening velocity while the ability to sustain load remained depressed. We conclude that the recuperative potential of myocardium damaged by pressure overload is adequate provided congestive heart failure has not occurred. Heart failure produces a persistent reduction in force-generating ability of the myocardium. Hypertrophy due to pressure overload, with or without CHF, leads to cardiac catecholamine depletion which is not readily reversed by relief of the overload.

MYOCARDIAL contractile function and function of the cardiac sympathetic system are impaired when ventricular hypertrophy and congestive heart failure result from a pressure overload on the heart.4,5 There is well established therapy to relieve the pressure overload; for example, systemic hypertension can be treated by pharmacological means and aortic stenosis can be relieved by cardiac surgery. However, relatively little is known of the potential for recovery of contractile function and repletion of myocardial catecholamine stores following relief of a pressure overload, despite the clinical relevance of such information for the correct timing of therapeutic interventions.6-8 Even less is known of factors that may determine the potential for return of contractile function and norepinephrine stores. A recent study8 has established that the contractile defect of hypertrophy due to pressure overload without heart failure, produced by experimental pulmonic constriction, is totally relieved after relief for approximately 4-5 weeks from the pulmonic stenosis.

The mechanics of cat papillary muscle can be described in a manner similar to that employed for skeletal muscle.11 It has been shown that the variation in mechanical muscle function is small within different groups of cats, thus functional parameters from different groups can be compared quantitatively.12 Since it is possible by constriction of the pulmonary artery in the cat to produce right ventricular hypertrophy with and without overt congestive heart failure,1 a source of myocardium from hypertrophied and failing hearts is available. In addition, surgical reversal of

From the Section of Cardiology and the Section of Cardiac and Thoracic Surgery, Temple University Health Sciences Center, Philadelphia, Pennsylvania.

Supported by the Southeastern Pennsylvania Heart Association and by Grants ST01-HL 05712 and 3RO1-HL 17631 from the National Institutes of Health. This work was completed during the tenure of fellowships granted to Dr. Coulson by the Medical Research Council of Canada and the Canadian Heart Foundation.

Dr. Bove is an Established Investigator, American Heart Association.

Dr. Yazdanfar's present address is: Albert Einstein Medical Center, Northern Division, Philadelphia, Pennsylvania 19141.

Received July 28, 1975; accepted for publication August 26, 1976.
The effect of indomethacin blockade of prostaglandin synthesis on blood pressure of normal rabbits and rabbits with renovascular hypertension.
J C Romero and C G Strong

Circ Res. 1977;40:35-41
doi: 10.1161/01.RES.40.1.35

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/40/1/35

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/